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Effects of sex and TNFα on adiposity, glucose tolerance and plasma adipokine levels in high fat diet-fed mice

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Table of Contents

Summary

Effects of sex and TNFα on adiposity, glucose tolerance and plasma adipokine levels in high fat diet (HFD)-fed mice

TNFα-deficient male mice show improved insulin sensitivity (IS), implicating TNFα in insulin resistance (IR). We extend previous results by investigating the effect of female sex on the protective effect of TNFα-deficiency on HFD-induced body weight (BW) gain and IR in mice.

Male and female TNFα KO and wild type (WT) mice were fed a HFD for 10 weeks (wk). BW gain, adipose tissue (AT) mass, intra-abdominal (IA) and subcutaneous (SC) fat mass, glucose tolerance and levels of fasting glucose, insulin, leptin and resistin before (wk 0) and after 5 and 10 wk of HFD feeding were measured.

From wk 1 of HFD access, lack of TNFα decreased BW gain in male but not female mice. Total AT as well as IA and SC fat mass were lower in KO than in WT and in female than in male mice. Fasting glucose was lower in KO than WT males but not females and lower in WT females than in males. Glucose tolerance was lower in KO than WT males in wk 0 and higher in females than males throughout. Females had lower plasma leptin and insulin levels than males on HFD and lack of TNFα had no effect on these hormones. Females and WT mice had higher plasma resistin levels than males and KO mice, respectively.

We demonstrate that the effect of TNFα-deficiency on BW gain and fasting glucose is sexually dimorph. TNFα and sex both affect adiposity and plasma adipokine levels and might also affect IS.

TNFα, sex, body weight, adipose tissue, glucose tolerance, insulin, leptin, resistin

Zusammenfassung

Einfluss des Geschlechts und TNFα auf Fettleibigkeit, Glukosetoleranz und Plasma-Adipokinwerte in fettreich gefütterten Mäusen

TNFα-defiziente männliche Mäuse zeigen eine erhöhte Insulinsensitivität (IS), was TNFα mit Insulinresistenz (IR) assoziiert. Wir untersuchen den Einfluss des weiblichen Geschlechts auf den protektiven Effekt von TNFα-Defizienz gegenüber Gewichtszunahme (GWZ) und IR verursacht durch fettreiche Diät (HFD).
Männliche und weibliche TNFα KO- und wildtyp-Mäuse (WT) wurden während 10 Wochen mit HFD gefüttert. Die GWZ, die Fettgewebsmasse, intra-abdominales (IA) und subkutanes (SC) Fett, Glukosetoleranz und gefastete Plasmaglukose-, Insulin-, Leptin und Resistinwerte wurden vor (wk 0) und 5 und 10 Wochen nach dem Wechsel auf fettreiche Diät gemessen.
Bereits in wk 1 der HFD, reduzierte TNFα-Mangel die GWZ in männlichen, aber nicht in weiblichen Mäusen. Sowohl die Gesamtfettmasse, als auch IA und SC Fett waren niedriger in KO verglichen mit WT und weiblichen verglichen mit männlichen Mäusen. Bei männlichen aber nicht in weiblichen Mäusen hatten KO Tiere tiefere Plasmaglukose als WT und WT Weibchen tiefere als Männchen. Die Glukosetoleranz war in wk 0 niedriger in KO als in WT Männchen und immer höher in Weibchen als in Männchen. Weibchen hatten niedrigere Leptin- und Insulinwerte als Männchen auf HFD, TNFα hatte keinen Einfluss auf diese Hormone. Weibchen hatten höhere Resistinwerte als Männchen und WT als KO Mäuse.
Wir zeigen, dass der Effekt von TNFα-Defizienz auf die GWZ und gefastete Glukosewerte sexuell dimorph ist. TNFα und das Geschlecht beeinflussen beide die Fettleibigkeit und Plasma-Adipokinwerte und möglicherweise auch die IS.

TNFα, Geschlecht, Körpergewicht, Fett, Glukosetoleranz, Insulin, Leptin, Resistin

1. Introduction

1.1. Obesity as inflammatory state

Obesity has been shown to be linked to insulin resistance and the metabolic syndrome through chronic, low-grade inflammation. This inflammation is characterized by the migration of bone marrow-derived macrophages into obese adipose tissue and the subsequent production of pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-6 (IL-6), IL-8 and many others (reviewed in (13)).

TNFα is a pro-inflammatory cytokine that plays a complex role in the response to injury and infection, in angiogenesis, apoptosis and other physiological processes. In the 19th century, an endogenous substance was observed to cause tumor regression in cancer patients with bacterial infection. This substance was found to be released by macrophages in response to the lipopolysaccharide derived from bacterial cell walls and was termed tumor necrosis factor (TNF). Later it was discovered that TNF is identical with the hormone cachectin that is associated with cachexia in cancer or prolonged infection (reviewed in (7)).

There are two forms of TNF, the 26 kDa transmembrane protein (mTNF) and the soluble, 17 kDa form of TNFα (sTNF) which is formed from mTNF by the TNFα converting enzyme (TACE) (6). sTNF is considered to be the biologically active form of TNFα. Over the last 15 years, evidence accumulated that TNFα plays a role in the development of the insulin resistance and type-2-diabetes (T2D) accompanying obesity. Mice heterozygous for TACE have been shown to have improved insulin sensitivity (43). Further, mice lacking TNFα gain less body weight and accumulate less body fat than wild type (WT) controls (50), and they have improved glycemic and lipid profiles. When mice lacking TNFα are placed on a high-fat diet (HFD), they become less obese than WT controls and have reduced plasma insulin and decreased insulin resistance (48).

Two TNFα-receptors, the 55 kDa receptor (p55) and the 75 kDa receptor (p75) have been described (1). In obese human subjects, the production of p75, but not p55, is increased in adipose tissue (23). Further, only the genetic deficiency of the p75 receptor has been shown to lead to increased insulin sensitivity, whereas mice lacking both receptor types (p55 and p75)

showed decreased insulin sensitivity compared to WT animals. This indicates that both receptors have different physiological functions (42)

TNFα is mainly produced by macrophages, but also by a broad variety of other cell types such as lymphoid cells, mast cells, endothelial cells and adipocytes (reviewed in (7)). TNFα mRNA is over-expressed in adipose tissue of genetic (e.g., the db/db, ob/ob or tub/tub mice and the fa/fa rat) and dietary-induced obesity models (25; 52). In inflamed adipose tissue of obese animals and humans, TNFα is mainly produced from bone marrow-derived macrophages (14). In contrast to the clear evidence for TNFα over-expression in adipose tissue of obese individuals, it is questionable whether circulating TNFα is also increased in obesity.

Treatment of cultured cells with TNFα showed that it impairs insulin signaling and can therefore lead to insulin resistance. Several molecular mechanisms appear to be involved in this effect: 1) The inhibition of the insulin receptor tyrosine kinase activity (24) that impairs insulin action, 2) the phosphorylation and activation of the protein tyrosine phosphatase SH-PTPase that leads to the termination of insulin action (2) and 3) the down-regulation of adipocyte gene expression of the glucose transporter-4 (GLUT4) and several insulin signaling proteins (e.g., insulin receptor substrate-1 (IRS-1) (41; 45). That adipose tissue TNFα over-expression contributes to insulin resistance was first suggested by the demonstration that the administration of TNFα in rats causes insulin resistance (31). Meanwhile several groups showed that neutralization of TNFα in vivo reverses both hepatic and skeletal muscle insulin resistance (8; 9; 12). Animals lacking TNFα function display improved insulin sensitivity in models of diet, genetically (ob/ob) or chemically (gold-thioglucose (GTG)) induced obesity (48; 50). The improved insulin sensitivity in these models is reflected in a reduction in circulating triglyceride levels, lower fed plasma leptin and lower fed and fasted plasma insulin levels. In mice fed a HFD for 4 weeks, Bouter et al. (10) found more subcutaneous fat in TNFα KO animals than in corresponding WT animals. An increase in subcutaneous vs. mesenteric adipose tissue is well known to be associated with increased insulin sensitivity (reviewed in (15)). Liver fat was also significantly reduced in TNFα KO animals (10).

1.2. Sex differences in obesity

The importance of sex differences has long been neglected in basic biomedical research and clinical trials, and female subjects still remain underrepresented, despite the strong evidence for sex differences in many basic metabolic processes as well as in the incidence of many diseases. Sex differences in the expression of thousands of genes have been observed (49), including those genes important for metabolic function (35). Sex differences have also been found in insulin resistance, body composition, energy balance and serum levels of adipokines, such as resistin, adiponectin and leptin (reviewed in (18; 19)). Visceral adiposity is a risk factor for the development of insulin resistance and T2D in both sexes. Male sex, however, is associated with increased visceral adipose tissue compared to females (18; 33); and this seems to be linked to elevated plasma insulin, free fatty acid (FFA) and triglyceride levels. Nonalcoholic fatty liver disease has also been shown to be linked to male sex, obesity and diabetes (reviewed in (18)). Estrogens appear to have beneficial effects on insulin sensitivity (40) through different mechanisms. Geary and Asarian showed that chronic estradiol treatment mimicking the regular 4 day estrous cycle in rats normalized body weight gain after ovariectomy, and it did so at least in part by increasing glucagon's and cholecystokinin's (CCK) satiating action (4; 5; 16; 17). Positive effects of estradiol on insulin and glucose homeostasis, adipose tissue metabolism, obesity, body composition or pro-inflammatory markers have also been described (reviewed in (18; 40)).

1.3. Aim/Hypothesis

Various studies in male individuals have shown a beneficial effect of TNFα-deficiency on insulin sensitivity in different rodent models of obesity, but the effect of TNFα-deficiency in females has not been examined yet. Estrogens influence inflammatory processes and insulin sensitivity (reviewed in (18; 40)) and might therefore also modify the beneficial effect of TNFα-deficiency in obesity-induced insulin resistance. The aim of this thesis was to examine this issue. More specifically, we investigated whether the beneficial effect of genetic TNFα-deficiency observed in male mice is as pronounced in females.

We used the model of HFD-induced obesity in mice, which has been shown to be a robust model to study impaired glucose tolerance and early T2D (39; 51). We examined the effect of sex and TNFα-deficiency on the development of body weight, adiposity, glucose

tolerance and plasma adipokine levels during 10 weeks of HFD feeding. A computer-tomographic (CT) scanner for laboratory animals (21) was used for adiposity measurements. This allowed for the longitudinal assessment of body composition of the same animals and for comparison association with the accompanying metabolic changes and changes in circulating adipokine levels in the course of the experiment.

2. Materials and Methods

2.1. Animals & housing

Subjects were TNFα KO male (n=10, male TNFα KO, koM) and female (n=8, female TNFα KO, koF) mice and corresponding wild type male (n=10, male WT, wtM) and female (n=10, female WT, wtF) controls bred in our own facility from breeding pairs originally purchased from Jackson Laboratory, USA (TNFα KO: stock no. 003008) and from Charles River, Germany (C57/BL6 stock no. 000664). At 6-8 weeks of age, the mice were single housed in Makrolon® type III cages (Indulab, Gems, Switzerland) on pine wood chip bedding (Lignocel hygienic animal bedding, IRS, Rosenberg, Germany) in a room kept on a 12 h light : 12 h dark cycle (lights on at 09:00 am), with 22 ± 2 °C and 60 % humidity. Mice received fresh chow (Provimi Kliba AG # 3436, Kaiseraugst Switzerland) every day in the third hour of the light phase (between 11:00 and 12:00 am); water was available ad libitum throughout. The mice were adapted to these housing conditions and to handling for 1-3 weeks before the experiment started. Unless otherwise noted, body weight was measured (to the nearest 0.1g) three times per week, and food intake was measured twice a week (to 0.01g) Average daily food intake was calculated from those measurements. Because the mice were food deprived for body adiposity assessments and blood samplings (see below), the food intake was not measured in the weeks when these measurements were performed (week 0, 5 and 10).

2.2. High-Fat Diet (HFD) feeding

Baseline measurements were taken at 9 weeks of age. One week later, at 10 weeks of age the mice were put on high-fat diet (HFD, 60% kcal from fat (lard) Provimi Kliba AG # 2127, Kaiseraugst, Switzerland) containing 35.5, 15.5 and 24 % weight fat, carbohydrate and protein respectively, with an estimated metabolizable energy content of 22 kJ/g. Fresh food was given every day and water was available ad libitum. During the first week on HFD, food intake was assessed daily. Thereafter, food intake was measured twice a week

2.3. Measurement schedule

For these baseline measurements at 9 weeks of age (wk 0) we performed an intraperitoneal (IP) glucose tolerance test (GTT) and collected venous blood on two consecutive days. After

one day without measurements we also performed whole-body computer-tomography (CT) scans. The same measurements were performed again in wk 5 and 10 after the switch to HFD.

2.3.1. Glucose tolerance test (GTT)

Mice were food deprived for 12 h (last 2 h of the dark phase and 10 h into the light phase) and the test was performed 2 h before dark onset. Blood was taken from the tip of the tail and blood glucose concentration was measured using a glucose meter (Accu-Chek Aviva, Roche Diagnostics AG, Rotkreuz, Switzerland). After the baseline (0 min) measurement, glucose 2 g/kg (20% glucose solution, "Glucosum" Grosse Apotheke Dr. G. Bichsel AG, Interlaken, Switzerland) was injected IP and blood glucose was measured 15, 30, 45, 60 and 120 min later. Food was returned after the last blood glucose reading.

2.3.2. Venous blood collection

Venous blood was collected from un-anesthetized mice. The mice were immobilized by placing them head first in a falcon tube of which the tip of the cone was cut off and the saphenous vein was exposed and punctured with an 18 gauge needle (Braun, Melsungen, Germany). The blood (~50µl) was collected in a microvette (Sarsted Microvettes CB300) coated with Li-Heparin as anticoagulant and centrifuged (10.000 g, 8 min, 4 °C) (Biofuge fresco, Heraeus, Kendro Laboratory Products AG, Zurich, Switzerland), the plasma was collected and stored at -20°C until further processing.

2.3.3. Computer tomography (CT) scans

Whole-body CT scans were obtained from anaesthetized mice (Isoflurane, Attane, Provet, Lyssach, Switzerland) using Micro CT LaTheta^{TM} CT scanner (Aloka Co. Ltd. Japan) at 1mm pitch, fast speed and high voltage in the face up and head front posture. Anesthesia was induced in a small acrylic box using a flow 400 ml/min O_2 with 5 % isoflurane and maintained in the scanner via a nose cone providing 100 ml/min O_2 with 1 % isoflurane. Aloka software estimates the volumes of adipose tissue, bone, air and soft tissue according to their X-ray density, distinguishes intraabdominal (IA) and subcutaneous (SC) fat based on detection of the abdominal muscle layers, and computes adipose tissue weight using a density factor of 0.92g/cm^3. Images were manually corrected if necessary and IA and SC fat values were obtained. The automatically calculated fat mass values were later divided by 0.92 to correct for underestimation (see (21)).

2.3.4. Plasma hormone measurements

Plasma concentrations of insulin, resistin and leptin were measured by multiplex assay (Linco Research, St. Charles, MO, USA; minimal detectable concentrations = 3.7, 1.6 and 24.9 pg/ml for insulin, resistin and leptin, respectively). Measurements were done in duplicates, and samples which had within assay coefficients of variations > 20% were excluded. For 13 samples single values were used because the sample volume was not sufficient for duplicate measurements. Because of technical problems, the wk 10 leptin values are not presented.

2.3.5. PCR verification of genotypes

To confirm the mice's correct genotypes, tail samples were taken after sacrifice and DNA was analyzed using the PCR protocol of Jackson Laboratories (USA). The primer used were oIMR4182 = TAG CCA GGA GGG AGA ACA GA (common primer), oIMR4183 = AGT GCC TCT TCT GCC AGT TC (wild type reverse primer) and oIMR7297 = CGT TGG CTA CCC GTG ATA TT (mutant reverse primer) (26).

2.4. Statistics

A robust statistical approach was adopted to increase statistical power (11; 47). Logarithmic or inverse transformations were used as required to improve normality. In addition, to reduce the influence of extreme values, data were converted to standard scores using the median absolute deviate method, and standard scores with absolute values > 1.96 (i.e., $P < 0.05$) were excluded. Data were then analyzed with two-way ANOVA and sex and genotype as main factors (Sigma stat version 3.5, Systat Software, San Jose, CA, USA) or, if transformations did not lead to normality, an one-way ANOVA on ranks followed by non-parametrical Mann-Whitney U tests. ANOVA were followed up with the sequentially-rejective Bonferroni test (22). Four post-hoc comparisons were tested: (1) male WT vs. male TNFα KO, (2) female WT vs. female TNFα KO, (3) male WT vs. female WT and (4) ΔM vs. ΔF (= male TNFα KO – male WT vs. female TNFα KO – female WT). Data are reported as means ± standard error of the mean (SEM) or as median ± interquartile range. The standard error of the difference (SED) generated by parametric analyses is given to indicate experiment-wide residual variability. The criterion for statistical significance was $P < 0.05$.

To determine the AUC values from the GTT glucose measurements, the area under the curve with respect to the increase from baseline was calculated (37).

The energy intake data except wk -1, 6 and 7, the AUC of the GTTs in wk 0 and wk 5, the total adipose tissue, IA and SC fat masses in wk 0, the plasma resistin concentrations in wk 5 and wk 10, and all insulin concentration data were normally distributed and therefore analyzed with parametric tests as described. Body weight data, fasted glucose levels in wk 5, total adipose tissue measures in wk 5 and wk 10, SC fat in wk 10, % SC fat in wk 5 and wk 10 underwent inverse transformation. IA fat in wk 10 and resistin data in wk 0 were transformed logarithmically. Body weight gain data, energy intake data in wk -1, 6 and 7, AUC in wk 10, IA and SC fat in wk 5, all % IA fat and wk 0 of %SC fat were not normal and equally distributed.

3. Results

3.1. Body weight gain (Figure 1)

Genetic TNFα-deficiency resulted in reduced body weight gain in male mice throughout the experiment, starting in the first week of HFD feeding (H(3) = 18.1, 21.1 and 25.4 for wk 1, 5 and 10, respectively, all Ps < 0.01), but not in female mice. Although the ANOVA on ranks revealed an overall significance, there was no significant difference in body weight gain in females at any time (U = 28, 243 and 224 for wk 1, 5 and 10, respectively). The genotype difference in weight gain was significantly greater in males than in females from the first day on HFD (H(3) = 18.1, 21.1 and 25.4 for wk 1, wk 5 and wk 10, respectively, Ps < 0.01; U = 203, 243 and 224 for wk 1, wk 5 and wk 10, respectively).

Male WT mice gained more weight than female WT mice from the first week on HFD (H(3) = 18.1, 21.1 and 25.4, for wk 1, wk 5 and wk 10, respectively, Ps < 0.01; U = 56, 70 and 70 for wk 1, wk 5 and wk 10, respectively).

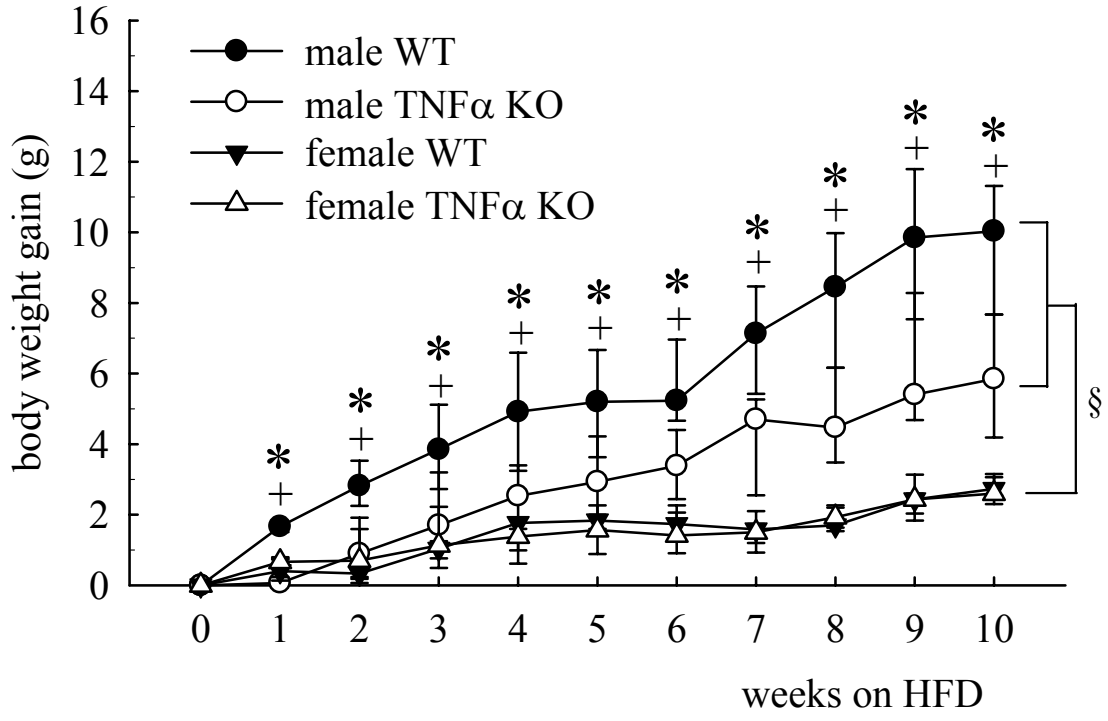

Figure 1: Cumulative body weight gain; Data are means ± SEM of 8-10 mice/group. * male TNFα KO < male WT, P < 0.05; $^{+}$ male WT > female WT, P < 0.01; § ΔM > ΔF, P < 0.01

3.2. Energy Intake (Figure 2)

Male TNFα KO mice ate more than male WT mice. This difference in energy intake was already significant prior to the switch to HFD ($F(1, 24) = 4.0$ for wk -2, $P < 0.05$. $H(3) = 20.1$ for wk -1, $P < 0.05$; $U = 59$). Also, when fed the HFD, the TNFα KO males overall tended to eat more than the WT mice and this difference was significant in wk 2, 7 and 9 on HFD ($F(1, 28$ and $1, 29) = 17.9$ and 17.1 for wk 2 and 9, respectively, $Ps < 0.01$. $H(3) = 13.5$ for wk 7, $P < 0.01$; $U = 8.5$). In the females, no genotype difference in energy intake was observed except for wk 2, when the TNFα KO females ate more than the WT females ($F(1, 28) = 17.9$, $P < 0.01$). The males tended to have a greater genotype difference in energy intake than the females, but this difference between sexes was only significant in wk 9 ($F(1, 29) = 40.2$, $P < 0.01$).

Male WT mice ate more than female WT mice. This difference was already present prior to the switch to HFD in wk -1 ($H(3) = 20.1$, $P < 0.01$; $U = 2$) and continued in wk 1 ($F(1,33) = 38.6$, $P < 0.01$) as well as in wk 6, 8 and 9 of HFD feeding ($H(3) = 15.4$ for wk 6, $P < 0.01$; $U = 71$. $F(1, 30$ and $1, 29) = 22.4$ and 40.2 for wk 8 and 9, respectively, $Ps < 0.05$)

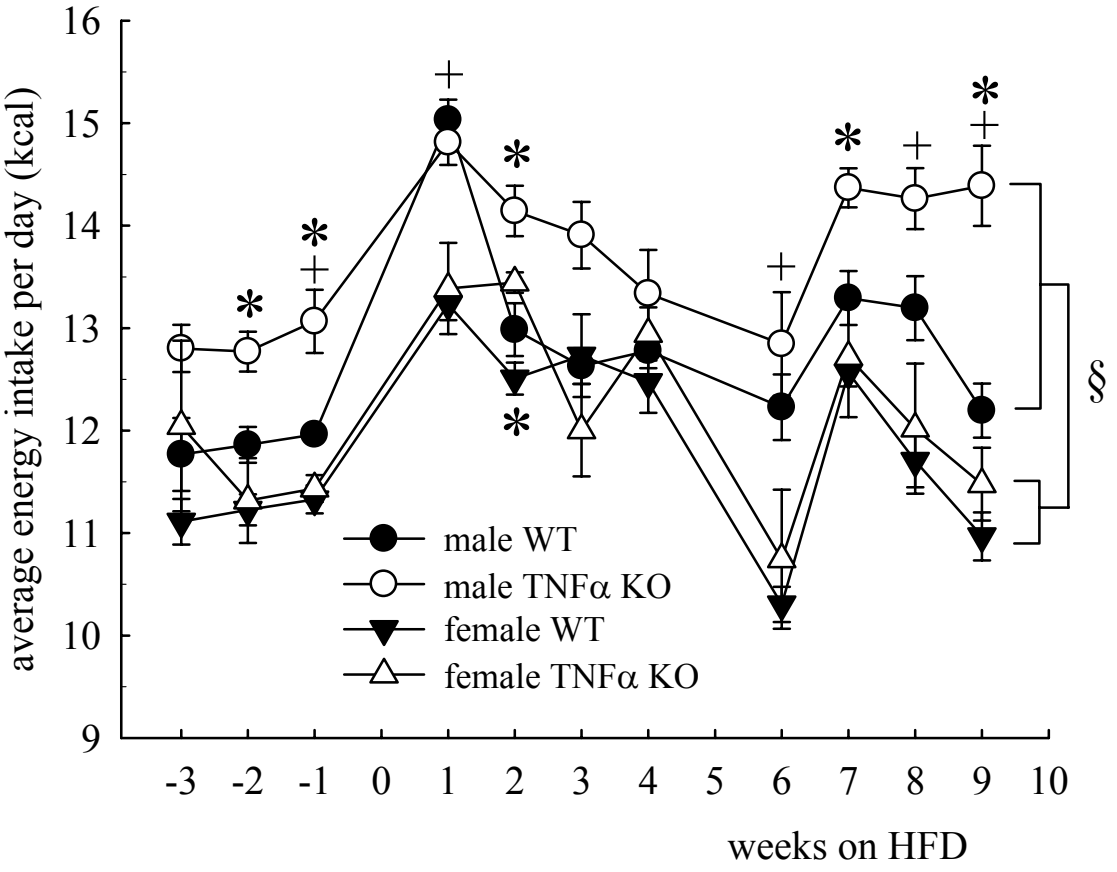

Figure 2: Energy intake; Data are shown as means ± SEM. * TNFα KO > WT, $P < 0.05$; $^{+}$ male WT > female WT, $P < 0.05$; § $\Delta M > \Delta F$, $P < 0.01$

3.3. Adiposity (Figures 3a-3c)

Genetic TNFα-deficiency resulted in reduced total adipose tissue mass in both sexes in wk 0 and wk 5, but not significantly in wk 10 ($F(1,30$ and $1,29) = 52.0$ and 26.2 for wk 0 and wk 5, respectively, $Ps < 0.05$). TNFα-deficiency also reduced IA fat pad mass at all time points and in both sexes, except for wk 5 in females ($F(1,31$ and $1,29) = 47.7$ and 18.1 for wk 0 and wk 10, respectively, $Ps < 0.01$; $H(3) = 25.3$ for wk 5 in males, $P < 0.01$; $U = 10$). Furthermore, TNFα-deficiency reduced SC fat pad mass in wk 0 in both sexes and in wk 5 in females, but not in wk 5 in males and wk 10 for both sexes ($F(1,30) = 45.0$ for wk 0, $Ps < 0.01$; $H(3) = 23.2$ for wk 5 in females, $P < 0.01$; $U = 38$). Also, the genotype differences in total adipose tissue mass and SC fat pad mass were greater in males than in females in wk 5, although male WT did not have significantly more SC fat in wk 5 than male KO. This was probably due to the greater variability in the data of SC fat in male mice in wk 5. No significant genotype differences between sexes were seen in total and SC fat in wk 1 and wk 10 or in the IA fat at

any time point (total adipose tissue: $F(1,29) = 3.8$ for wk 5, $P < 0.01$; SC fat pad: $H(3) = 23.2$ for wk 5, $P < 0.01$)

Male WT mice and female WT mice had similar SC fat pad masses in wk 0, whereas the IA fat mass was greater in male than in female WT mice (IA fat: $F(1,31) = 24.0$ for wk 0, $P < 0.01$). The IA fat mass difference, however, was not big enough to account for a significant sex difference in total body fat. In wk 5 and 10, male WT mice had greater total fat pad masses and also greater SC and IA fat pad masses than female WT mice (total fat: $F(1,29$ and $1,30) = 97.8$ and 75.0 for wk 5 and wk 10, respectively, $P < 0.01$; IA fat: $H(3) = 25.3$ for wk 5, $P < 0.01$; $U = 54$; $F(1,29) = 205.4$ for wk 10, $P < 0.01$; SC fat: $H(3) = 23.2$ for wk 5, $P < 0.01$; $U = 80$; $F(1, 29) = 117.9$ for wk 10 , $P < 0.01$),

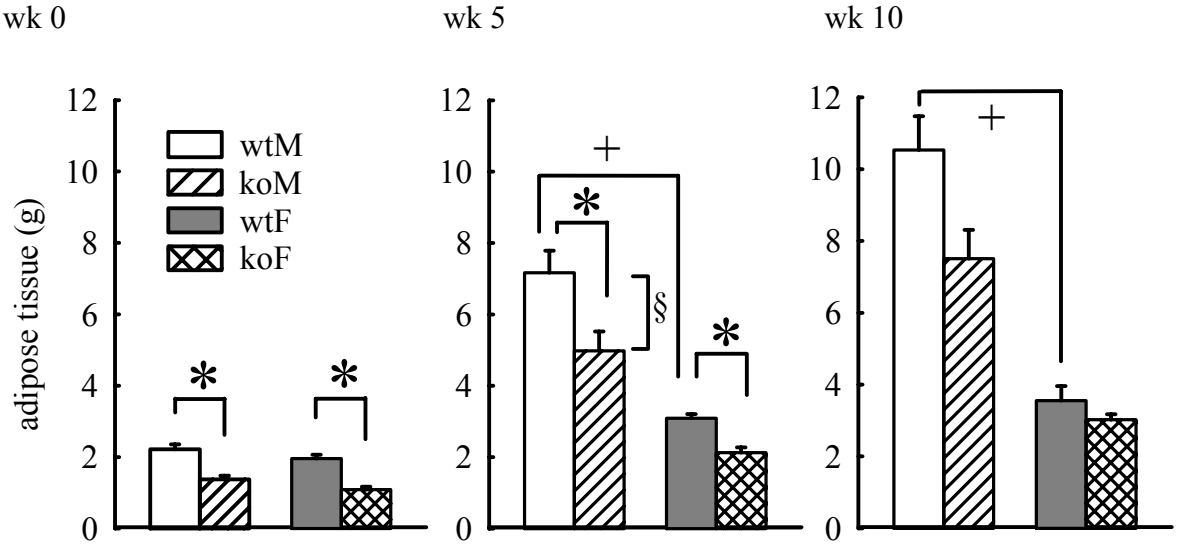

Figure 3a. Total adipose tissue; **wk 0:** * KO < WT, $P < 0.01$. **wk 5:** * KO < WT, $P < 0.05$; $^{+}$ male WT > female WT; § $\Delta M > \Delta F$, Ps < 0.01. **wk 10:** $^{+}$ male WT > female WT, $P < 0.01$. Data are shown as means ± SEM. See text for further details.

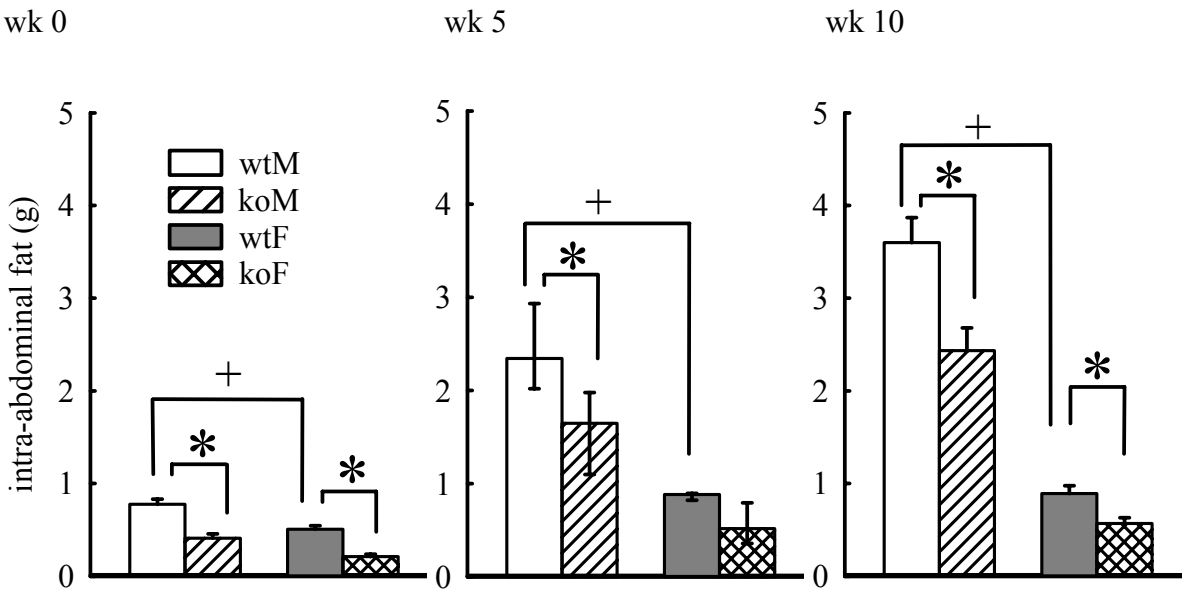

Figure 3b: Intra-abdominal fat; $^{+}$ male WT > female WT, Ps < 0.01. **wk 0:** Data are shown as means ± SEM; * KO > WT, Ps < 0.01. **wk 5:** Data shown as medians ± interquartile range; * male TNFα KO < male WT, , P < 0.01. **wk 10:** Data are shown as means ± SEM; * KO < WT, P < 0.01

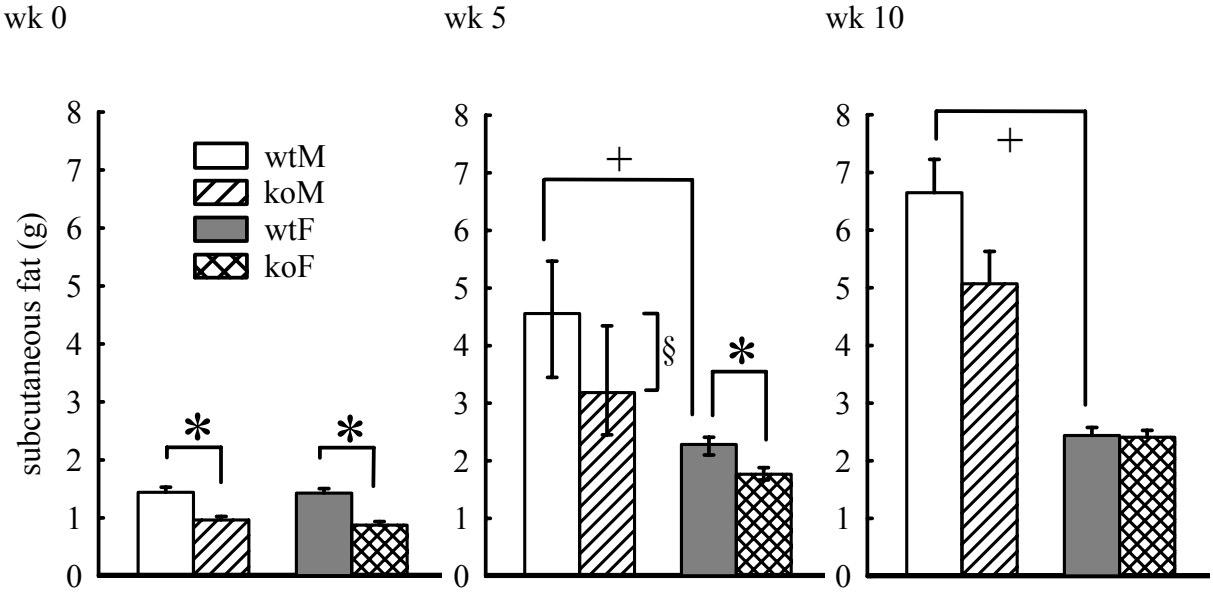

Figure 3c: Subcutaneous fat; **wk 0:** Data are shown as means ± SEM; * KO < WT, P < 0.01; **wk 5:** Data shown as medians ± interquartile range; * female TNFα KO < female WT, $^{+}$ male WT > female WT, § ΔM > ΔF, Ps < 0.01; **wk 10:** Data are shown as means ± SEM; $^{+}$ male WT > female WT, P < 0.01

3.4. Body fat distribution (Figure 4)

TNFα-deficiency led to a relatively lower percentage of IA and higher percentage of SC fat in both sexes (% IA fat males: H(3) = 27.9, 31.1 and 29.4, for wk 0, 5 and 10, respectively, Ps < 0.01; U = 87, 4 and 0, for wk 0, 5 and 10, respectively; % IA fat females: H(3) = 27.9, 31.1 and 29.4, for wk 0, 5 and 10, respectively, Ps >; U = 0, 61 and 52, for wk 0, 5 and 10, respectively; % SC fat males: H(3) = 27.9 for wk 0, P < 0.01; U = 3. F(1, 32 and 1, 29) = 47.9 and 59.9, for wk 5 and 10, respectively , Ps < 0.01; % SC fat females: H(3) = 27.9 for wk 0, P < 0.01; U = 64. F(1, 32 and 1, 29) = 47.9 and 59.9, for wk 5 and 10, respectively, Ps < 0.01). This body fat distribution did not change in the course of the HFD feeding. The genotype difference was not significantly different between males and females.

WT female mice had significantly less % IA and more % SC fat compared to WT males. The sex difference in body fat distribution did also not change in the course of the experiment (% IA fat: H(3) = 27.9, 31.1 and 29.4, for wk 0, 5 and 10, respectively, Ps < 0.01; U = 80, 90 and 72 for wk 0, 5 and 10, respectively; % SC fat: H(3) = 27.9 for wk 0, P < 0.01; U = 0; F(1, 32 and 1, 29) = 174.1 and 271.0, for wk 5 and 10, respectively , Ps < 0.01

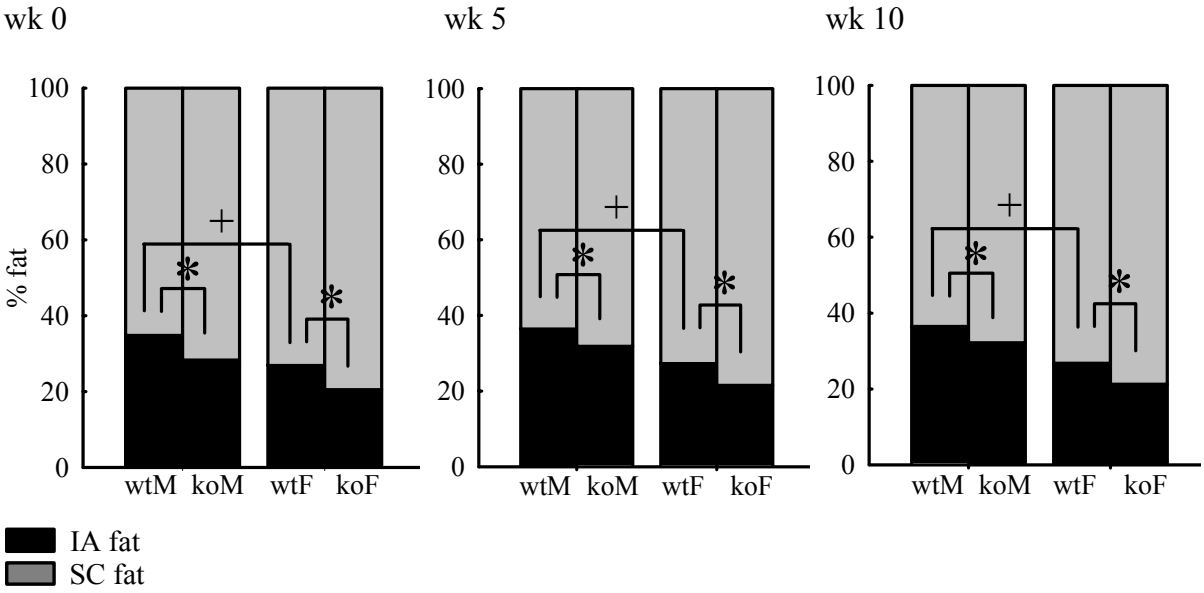

Figure 4. Body fat distribution; * KO < WT, Ps < 0.01; $^{+}$ male WT > female WT, Ps < 0.01

3.5. Fasting blood glucose levels and glucose tolerance (Figures 5 and 6)

Male TNFα KO mice had lower fasting blood glucose levels compared to male WT mice in wk 0, but higher glucose levels in wk 5 ($H(3) = 28.3$ for wk 0, $p < 0.01$; $U = 90$; $F(1,31) = 6.1$ for wk 5, $P < 0.01$). No genotype difference in the fasting glucose level was found for male mice in week 10 and at all time points in the female animals. Despite the lower fasting glucose level, TNFα KO mice had a greater AUC for glucose than male WT mice in the glucose tolerance test in wk 0 ($F(1,28) = 15.5$ for wk 0, $P < 0.01$), whereas the AUC was not significantly affected by TNFα-deficiency in both sexes in wk 5 and wk 10, not even by the significantly higher wk 5 fasting glucose level in the TNFα KO male animals compared to the WT males.

Female WT mice had lower fasting blood glucose levels than male WT mice at all time points ($H(3) = 28.3$ and 25.8 for wk 0 and wk 10, respectively, $Ps < 0.01$; $U = 0$ for both wk 0 and wk 10; $F(1, 31) = 99.1$ for wk 5, $P < 0.01$). Despite the lower baseline glucose levels of the WT females compared to WT males at all time points there was no significant difference between sexes in the AUC for glucose in the glucose tolerance test in wk 0. In wk 5 and 10, however, the AUC was greater in the WT males than in the WT females ($F(1,33) = 13.6$ for wk 5, $P < 0.01$; $H(3) = 8.1$ for wk 10, $P < 0.01$; $U = 10$).

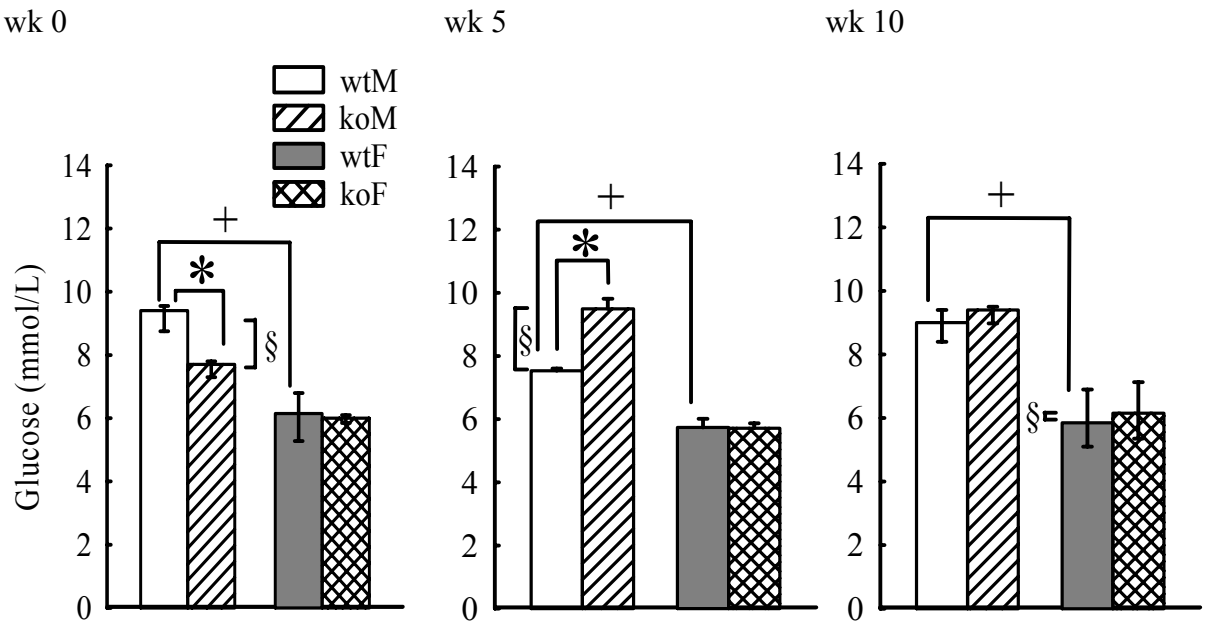

Figure 5: Fasting blood glucose level; $^{+}$ male WT > female WT, Ps < 0.01 **wk 0:** Data shown as medians ± interquartile range. * male TNFα KO < male WT, § ΔM > ΔF, Ps < 0.01 **wk 5:** Data are shown as means ± SEM. * male TNFα KO > male WT, § ΔM > ΔF, Ps < 0.01 **wk 10:** Data shown as medians ± interquartile range. § ΔM < ΔF, P < 0.05

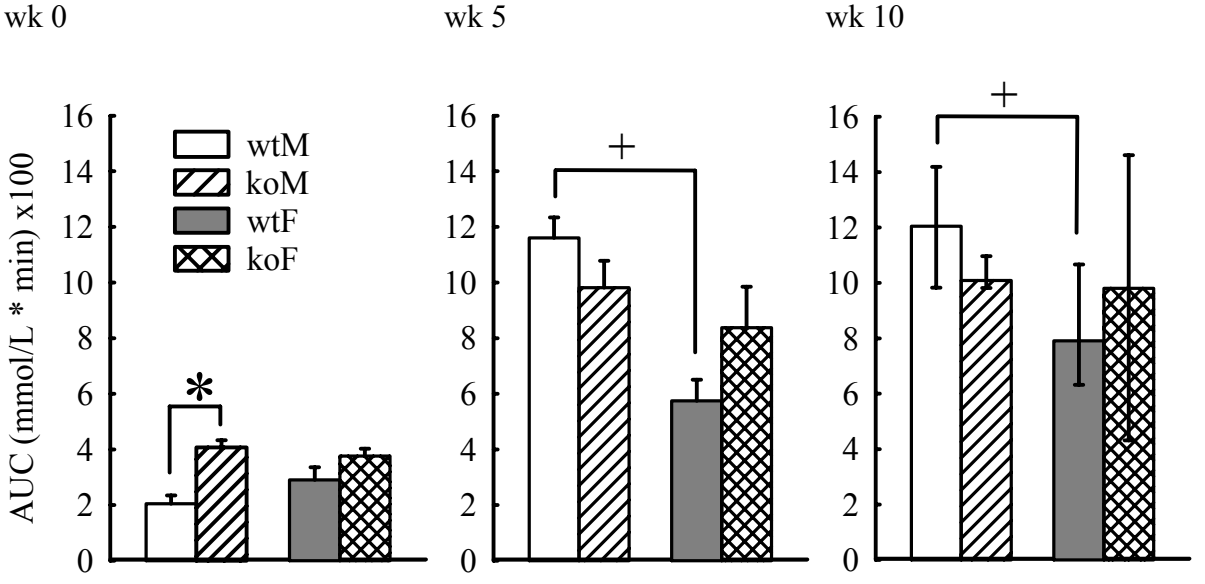

Figure 6: AUC for blood glucose during the ipGTT; **wk 0:** Data are shown as means ± SEM. * male TNFα KO > male WT, P < 0.01 **wk 5.** Data are shown as means ± SEM. $^{+}$ male WT > female WT, Ps < 0.01 **wk 10:** Data are shown as medians ± interquartile range, $^{+}$ male WT > female WT, Ps < 0.01

3.6. Plasma hormone levels

3.6.1. Leptin

Genotype did not significantly affect plasma leptin levels in wk 0 or wk 5. Because of technical problems with the assay, wk 10 leptin values could not be used. There was a tendency towards lower leptin levels in TNFα KO mice compared to WT mice in both sexes in wk 0 and in the males in wk 5, but these differences were not significant. The genotype differences were also not significantly different between males and females.

There was no sex difference in wk 0, but in wk 5 plasma leptin levels were higher in WT male than in WT female mice ($F(1,24) = 84.2$, $P < 0.01$)

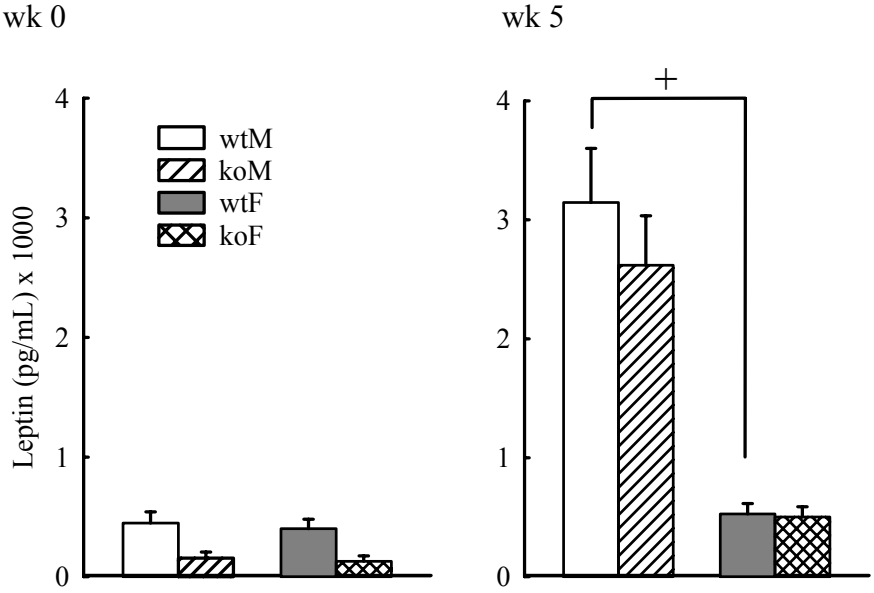

Figure 7: Plasma leptin levels; (10 wk data can not be shown because of technical problems with the assay). Data are means ± SEM. $^{+}$ male WT > female WT, $P < 0.01$

3.6.2. Resistin

In wk 0 TNFα KO male and female mice had lower fasting plasma resistin levels than WT mice of both sexes and in wk 5 this genotype difference was still present in female mice, but not in male mice ($F(1,27$ and $1,32) = 47.0$ and 4.7 for wk 0 and wk 5, respectively, $Ps < 0.01$). In wk 10, however, the lowering effect of TNFα-deficiency on plasma resistin was no longer apparent. There were no significant genotype differences between sexes in wk 0 and 10, but

the significant genotype difference in females led to a greater genotype difference in females than in males in wk 5 ($F(1,32) = 5.4$ for wk 5, $P < 0.01$).

Female WT mice had higher resistin levels compared to male WT mice in wk 0 and wk 5 ($F(1,27)$ and $F(1,32) = 36.0$ and 13.4 for wk 0 and wk 5, respectively, $Ps < 0.01$) . In wk 10, however, the sex difference was no longer seen.

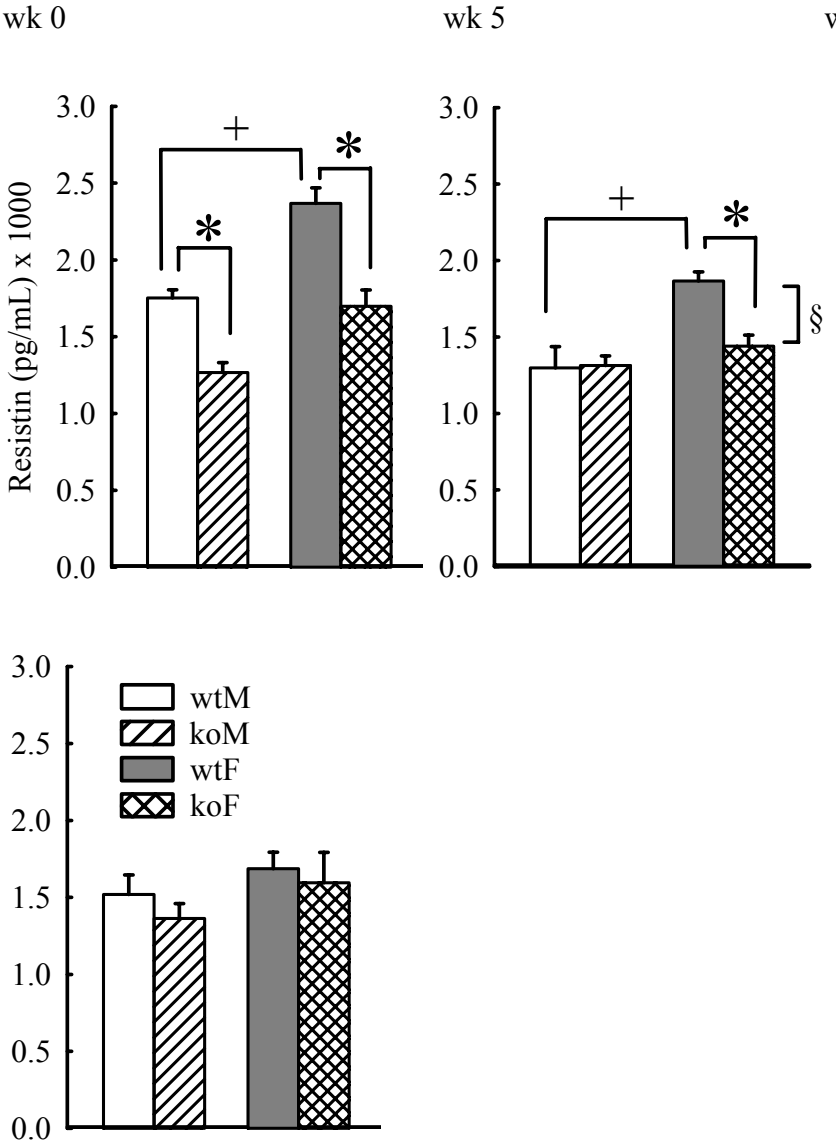

Figure 8: Plasma Resistin Levels; Data are shown as means ± SEM. **wk 0:** * KO < WT, $^{+}$ male WT < female WT, Ps < 0.01 **wk 5:** * female TNFα KO < female WT, $^{+}$ male WT< female WT, § ΔM < ΔF, Ps < 0.01 **wk 10:** No significant differences.

3.6.3. Insulin

Genotype did not affect plasma insulin levels at any time point. There was a tendency towards lower insulin levels in TNFα KO males and towards an inconsistent effect of TNFα-deficiency in females, but none of these differences reached statistical significance. There was also no significant genotype difference between the sexes.

Male WT mice tended to have higher plasma insulin levels than female WT mice at all time points, but the difference reached significance only in wk 10 ($F(1,23) = 10.8$, $P < 0.01$).

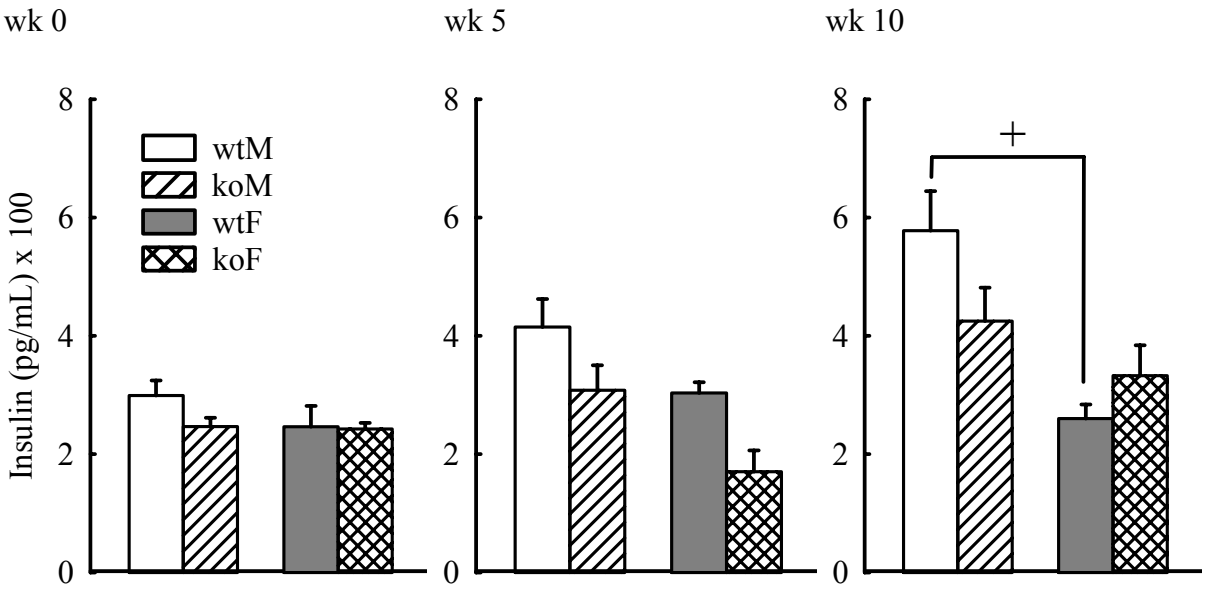

Figure 9: Plasma insulin levels; Data are shown as means ± SEM. **wk 10:** $^{+}$ male WT > female WT, $P < 0.01$

4. Discussion

Overweight and obesity constitute a major (or the most important) risk factor for T2D and other related diseases. Also undisputed is the existence of sex differences in the incidence of obesity, in regional body fat distribution, and in the resulting development of insulin resistance or cardiovascular disease. It has been amply demonstrated that genetic or pharmacologic removal of TNFα protects from insulin resistance in male subjects (10; 48; 50). In this experiment we revisited the protective effect of genetic TNFα-deficiency from HFD-induced overweight and insulin resistance and extended previous reports by examining, for the first time, (1) whether female mice are less susceptible to HFD-induced obesity and its metabolic consequences than male mice and (2) whether sex affects the beneficial effect of TNFα-deficiency. We confirm that the lack of TNFα leads to less adiposity, improved fat distribution towards more SC and less IA fat and to lower insulin, leptin and resistin levels, and report that (1) females show less weight gain, a more favorable fat distribution, altered hormone levels and less insulin resistance compared to males. Moreover, (2) in contrast to the males, there was no influence of TNFα on body weight gain or fasted glucose levels in female mice.

4.1. Body weight gain

The effect of TNFα deletion on body weight gain is sexually differentiated. That is, male WT mice gained significantly more weight than male TNFα KO mice, whereas there was no difference in weight gain between WT and KO female mice. This effect was present throughout the entire 10 wk of HFD feeding. Because most studies in TNFα KO mice only used male animals (10; 32; 48), it has not been noted before that TNFα-deficiency has no effect on body weight gain in female animals. There is only one study that looked at the effect of TNFα-deficiency on body weight gain in females. Interestingly, similar to what we observed here, female TNFα KO mice in this study showed no significant difference in weight gain compared to WT female mice, even though the obesity trigger was different (50), i.e., gold-thioglucose injections vs. HFD in the present study. Because of the lack of body weight gain in WT female mice on HFD, however, it is difficult to interpret these data as resistance to body weight gain conferred by the lack of TNFα in female mice.

One interesting observation in our study is that there was a significant difference between the WT and KO male mice already after 1 week of HFD feeding, i.e., much sooner than the effect obtained previously by Bouter et al. (10) under similar conditions, where a significantly greater weight gain in WT male mice compared to TNFα KO animals was only seen after 3 weeks of HFD. The experimental conditions were almost identical in both studies, with the only exception that the source of the TNFα KO animals was different. All animals used here were bred in our own SPF facility, but for the study of Bouter et al. (10) the original breeding pair was donated by Dr. M. Freudenberg (Max Planck Institute, Freiburg, Germany), whereas for our experiment, the original breeding pair was purchased from Jackson Laboratories USA. In any case, the present results suggest that already the first week of HFD feeding can be critical in determining the factors that influence body weight gain in male mice, but not in female mice. Since we did not study chow fed mice in parallel, it is unclear whether the effect of TNFα-deficiency on body weight gain was masked by age-related growth.

There is prior evidence that TNFα-deficiency has an effect on body weight gain during HFD feeding in male mice. Previous findings, however, are not entirely consistent with ours, probably because of several key differences in the experimental conditions. Thus, several important parameters were different between our experiment and previous studies: The age and body weight of the animals at the time of exposure to HFD were different, the control animals used, the fat content of the diet and the source of fat. In the study of Lee et al. (32) for instance, the mice were younger (6 vs. 9 wks in our study), but about 10 g heavier at the onset of the experiment. The strain B6/29SC vs. C57/BL6 was used for WT controls and the HFD contained 42 % vs. 60 % of fat and the source of fat was milk vs. lard. Nevertheless, after 5 wk of HFD feeding, the weight gain in KO HFD mice was less than in WT HFD mice, i.e. similar to our study, although the difference was not statistically significant (KO HFD: 5.2 ± 0.6 vs. WT HFD: 8.2 ± 1.9 g). Interestingly, within a similar time period of HFD feeding, the weight gain was 50% greater in magnitude than what we observed (8.2g vs. 5.3g in WT animals and 5.2g vs. 3.1g in TNFα KO animals). In another study (48), younger (4 vs. 9 wk old animals) and about 8 g lighter male mice were used, and similar to Lee (32) , C57/BL6 x 129 animals were used as WT controls. The body weight gain induced by a diet with a lower fat content than in our experiment (35 %) was significantly different in WT vs. KO mice only

after 12 wks of HF feeding, whereas in our studies, a significant weight gain difference in WT vs. KO was already observed from the first week on HFD.

In sum, it seems that the animals' age and/or body weight at which the HFD feeding is introduced plays a role in the effect of TNFα-deficiency on body weight gain. Animals at young age (4-6 weeks, (32; 48)) show greater weight gain and no effect of TNFα-deficiency on weight gain, whereas when animals are exposed to HFD at older age (8-9 weeks, (10), our experiment), TNFα-deficiency leads to less weight gain. Despite this lower weight gain, however, KO animals ate more than WT animals, showing that the reduced weight gain can not be due to decreased energy intake, but rather could be due to increased energy expenditure. Differences in energy absorption and metabolism could also be a reason for the genotype difference in body weight gain. Further metabolic studies need to be performed to answer this question.

4.2. Adiposity

4.2.1. Total adipose tissue, body adiposity

The gradual loss of the ability of TNFα-deficiency to significantly decrease adipose tissue mass suggests that TNFα influences adipose tissue primarily at the onset of obesity, but that other mechanisms may become more important after prolonged HFD feeding. However, because there was still a tendency towards smaller adipose tissue mass in TNFα KO animals in wk 10, it is also possible that the loss of significance was simply due to the increase in the variability of our adiposity data over time. On the other hand, similar to our findings, also Bouter et al. (10) did not observe differences in total white adipose tissue mass after 4 weeks on HFD, despite a significantly greater body weight in male WT mice compared to male TNFα KO mice. But this was earlier than in our experiments (4 weeks vs. 10 weeks of HFD). In our study, we still found a genotype difference in adiposity in both sexes in wk 5 on HFD. Because there was no week 0 data in the study of Bouter et al. (10), it remains unknown whether those animals also showed a genotype difference in adiposity prior to HFD exposure.

Another study in male animals (50) assessed body fat through carcass digestion by alcoholic potassium hydroxide hydrolysis. Consistent with our findings, this study found a higher percentage of body fat in WT animals compared to KO animals. It has to be noted,

though, that body fat determined through digestion includes also ectopic fat in organs, whereas CT scanning only measures adipose tissue depots.

Male mice were more susceptible to fat deposition and weight gain induced by HFD feeding than female mice, despite similar total body adiposity at the study onset. On one hand this might simply be because of age-related growth which is different in males and females (growth curves stock no. 003008 and 000664, Jackson Laboratories, US) and the sex difference in food intake; female mice ate significantly less than male mice. On the other hand, the protective effect of estrogens could have played an important role in the females. Thus, Riant et al. (40) found that body weight was significantly reduced be estradiol administration in ovariectomized mice put on HFD. Thus, the protective effect of estradiol may have prevailed and, hence, masked, any protective effect of TNFα-deficiency in our study.

The reducing effect of TNFα-deficiency on adipose tissue was bigger in males compared to females. These findings are consistent with our body weight gain data, i.e., with the observation that TNFα-deficiency failed to significantly reduce the body weight gain in female mice. It is possible that this sex difference in the effect of TNFα-deficiency on body fat was just proportional to the increased adiposity in males compared to the females. In any case, however, this difference in adiposity could be one important mechanism contributing to the beneficial effect of TNFα and female sex on insulin sensitivity.

4.2.2. Body composition, intra-abdominal fat and subcutaneous fat

In our study, female sex was associated with less metabolically unfavourable IA fat than male sex no matter of the kind of diet. This is consistent with many findings in animal and human studies (18; 19; 33). The size of the SC fat depot did not differ between male WT and female WT on chow. On HFD, however, males developed significantly larger SC fat pads than females. This is most likely due to the increased overall adiposity in males compared to females. If the IA fat and the SC fat were expressed as % adipose tissue, male mice showed more % IA fat and less % SC fat than females and this ratio did not change in the course of the experiment.

Several studies found larger epididymal fat in WT mice compared to KO mice in lean (10; 50) and obese mice ((10; 48) 22% increase). Ventre (50), however, did not report any

difference between chemically (GTG) induced obese TNFα KO and WT mice, whereas Uysal found no difference in lean animals. It has to be noted that the epididymal fat pad (48; 50) does not entirely contribute to the IA fat pad mass, which also includes mesenteric and retroperitoneal fat. Therefore, we can not directly compare our IA fat depot findings with changes in epididymal fat reported in the literature.

Similar to its effect on IA fat, TNFα-deficiency also decreased SC fat pad mass, but only in wk 0 in the males and in wk 0 and 5 on HFD in the females. There is only one study which also looked at SC fat pads (10), and these authors found more SC fat in KO compared to WT males after 4 weeks of HFD, which is different from our findings. This discrepancy is probably due to the greater overall adiposity in WT than in KO animals which was observed in our study, but not in the study of Bouter et al. (10). This difference presumably led to the greater fat pad mass in both IA and SC fat pads in our study. If the IA fat and SC fat were expressed as % adipose tissue, however, KO animals in both sexes showed less IA fat and more SC fat compared to WT.

The lack of TNFα entailed a beneficial fat distribution with smaller IA fat pads in both sexes. It is well established that the accumulation of IA fat is associated with insulin resistance in both sexes. The shift of fat distribution towards less IA fat could therefore be another mechanism by which TNFα-deficiency contributes to improved insulin sensitivity.

4.3. Fasting Blood Glucose and Glucose Tolerance

Low fasting blood glucose levels are usually considered to reflect high insulin sensitivity. The lower fasting blood glucose levels of male TNFα KO mice compared to male WT mice are therefore consistent with a beneficial effect of TNFα-deficiency on insulin sensitivity. Our findings in male mice also suggest, however, that the effect of TNFα-deficiency on fasting glucose changes in the course of HFD feeding. This might partially explain the differences in the effect of interference with TNFα signaling on fasting glucose found in the literature. The exact time course of this development and its biological relevance, however, need to be further investigated.

Lower fasting glucose in TNFα KO male animals were also found by Ventre et al. (50), who reported 10% lower fasting glucose levels in lean TNFα KO mice, but also a 14 % decrease in chemically (GTG) induced obese TNFα KO mice compared to WT mice after 28 weeks. These findings are consistent with observations by Bouter et al. (10), where lower fasting glucose levels in KO mice were found after 1 and 4 weeks on HFD. The latter study, however, did not investigate longer time periods. It is therefore impossible to know whether the reduction of fasting glucose by TNFα-deficiency would have persisted after prolonged HFD-feeding or would also have changed with time, similarly to what we found in the present study. In contrast, Lee et al. (32) observed that glucose levels tended to be higher in TNFα KO mice than in WT mice after 5 weeks of HFD. While this difference was not significant, it appears to be consistent with our findings in wk 5, when we found higher fasting glucose levels in TNFα KO male animals compared to females. Uysal et al. (48) observed no significant difference in fasting glucose levels between HFD-fed TNFα KO and WT animals. The TNFα KO animals tended to have lower glucose than WT mice until the age of 8 weeks, whereas at 12 weeks of age, the plasma glucose values were very similar. This is consistent with our findings in animals after 10 weeks of HFD, when we also did not observe a significant genotype difference anymore. Thus, the effect of TNFα-deficiency on fasting blood glucose levels seems to be situationally variable. The underlying mechanisms and the possible relationship to insulin sensitivity need to be examined.

We have no explanation for the relative glucose intolerance of male TNFα KO animals compared to male WT mice prior to HFD exposure, a phenomenon that is in contrast to all previous findings showing significantly (about 15%) greater glucose tolerance in animals lacking TNFα (48; 50). It is unlikely that the slightly, but not significantly, higher body weight of TNFα KO mice at this time point contributed to the low glucose tolerance because the adiposity and body composition data, (i.e., the fact that TNFα KO mice had less adipose tissue and less IA fat), would rather suggest an improved glucose tolerance.

Interestingly, the females showed a similar tendency towards a greater AUC in TNFα KO mice at all time points, but without significant difference. This prompts the speculation that in an overall relatively insulin sensitive state as in young animals prior to HFD exposure TNFα could actually have a positive effect on glucose tolerance. The possible mechanisms of

such an effect are enigmatic. If this speculation were true, however, only an obesity-promoting challenge of the organism, such as HFD exposure, might unveil the deleterious effect of endogenous TNFα on insulin sensitivity. Further studies are required to examine this idea.

Female mice had improved glucose homeostasis with lower baseline glucose levels and greater glucose tolerance compared to males. On the other hand, the presence or absence of TNFα alone had no effect on glucose metabolism in females under our conditions.

The improved fasting plasma glucose levels and glucose tolerance in females compared to males could be due to the lower body weight and adiposity on one hand, but also to the beneficial effect of estradiol on the other hand (40). Riant et al. (40) observed that estradiol protected ovariectomized mice from HFD-induced insulin resistance. The insulin action was still preserved after 1 month of HFD feeding and was reduced by 25% only after three months of HFD feeding. Because the females do not develop such a strong HFD-induced insulin resistance as males, a possible improvement by TNFα-deficiency may not be marked enough to be detected. Another study in humans (53) showed an improved glucose uptake by female muscle tissue compared to males which could also contribute to the lower plasma glucose levels and improved glucose tolerance in women compared to men (29; 53).

4.4. Plasma hormone levels

4.4.1. Leptin

Leptin levels tended to be lower in TNFα KO mice than in WT mice in both sexes. This genotype difference was not significant, but is consistent with the general view that circulating leptin levels reflect body adiposity (27) because the total adipose tissue mass showed a very similar pattern with KO mice having less adipose tissue than WT mice and males having similar adiposity as females prior to HFD. When put on HFD, TNFα KO males tended to have lower leptin levels than WT males, again correlating with the significantly lower adiposity in TNFα KO males. Ventre et al. (50) also did not find a significant decrease in mean leptin levels in TNFα KO GTG-obese compared to WT mice. In contrast, other studies found lower fasting plasma leptin levels in TNFα KO mice (10; 28). The difference in adiposity between TNFα KO and WT mice was probably not large enough to be reflected in a

significant difference in plasma leptin levels under our conditions. We also found no evidence that the presence or absence of TNFα could have a direct effect on leptin levels, independent of adiposity.

When put on HFD, male mice had higher circulating leptin levels than female mice. This was not observed before the diet switch, e.g. when the mice were still fed chow. The higher leptin levels in HFD fed male mice could simply be due to the increased adiposity seen in male mice compared to females. But there could also be a specific sex effect. Gui et al. (19) also found higher leptin levels in male than in female mice starting at 9 weeks of age. Similar findings were reported for rats (30; 34). A possible mechanism for the higher circulating leptin levels in males compared to females could be the lower leptin sensitivity of adipose tissue in males compared to females (36). In contrast to the situation in rodents, several human studies have revealed higher leptin levels in women than in men (18; 20; 27; 35; 44). This is consistent with the generally higher percentage of body fat of women compared to men and shows that the direct translation of findings from laboratory animals to humans is not always possible.

4.4.2. Resistin

TNFα KO mice showed lower resistin levels than WT mice prior to HFD feeding, whereas with prolonged HFD feeding and the development of overweight, the lowering effect of TNFα-deficiency on plasma resistin levels wore off. Previous studies in male mice found a correlation between circulating resistin and body fat in mice fed a high fat diet (38). Other studies found no genotype effect on plasma resistin levels in HFD-fed mice (10; 32). This is similar to our findings after 5 wk on HFD for the males and 10 wk for both sexes, when the lowering effect of TNFα-deficiency on plasma resistin levels was no longer apparent. Together with previous observations (10) of a genotype difference in circulating resistin in chow-fed but not in HFD-fed mice, our findings suggest that a lowering effect of TNFα-deficiency on plasma resistin levels is only visible in a lean state and diminishes with increasing obesity. Together with the lack of a significant difference in adiposity and the inability of TNFα-deficiency to produce a significant decrease in plasma resistin, our observations confirm previous findings suggesting that the lack of TNFα only partially protects against obesity-induced insulin resistance.

We observed higher resistin levels in female compared to male mice prior to and after 5 weeks on HFD (corresponding to 9 and 14 weeks of age), but after 10 wk exposure to HFD (19 weeks of age) the sex difference wore off. Similar findings were reported by Gui et al. (19). They observed sex differences in plasma resistin at 3 weeks of age, when male mice had higher levels than female mice. Thereafter the plasma resistin level progressively decreased in males and increased in females over 9 weeks to significantly higher levels than in males. Subsequently plasma resistin in females decreased again to levels similar to the male mice. Human studies also found higher resistin levels in women compared with men (44).

The higher resistin levels in females compared males do not support the hypothesis that resistin directly links obesity with insulin resistance (46) because females had lower adiposity and greater insulin sensitivity than males. This suggests that other mechanisms than adiposity contribute to the sex differences in plasma resistin levels and affect its influence on insulin sensitivity. Thus, the effects of sex hormones on circulating resistin levels and their exact relationship with insulin resistance remain unclear. Further studies are required to answer these questions.

4.4.3. Insulin

The presence or absence of TNFα did not significantly affect plasma insulin levels at any time point of our study. This does not match previous reports of significantly lower insulin levels in TNFα KO males compared to WT mice ((50) 67% lower in KO animals, (48) fourfold higher in WT animals, (10) about 20-50% lower in KO animals). We found a similar tendency, but the genotype difference in plasma insulin levels was not significant. This lack of a significant genotype difference may have been caused by the large variability of our data. Whether other factors contributed to this discrepancy remains unclear. Ventre et al. (50) did not find any genotype difference between chemically (GTG)-induced obese females until 32-34 weeks of age, which corresponds to our observations in the females.

No sex difference in circulating insulin was visible until wk 10, when WT male mice had higher plasma insulin levels than the corresponding females. Ahrén et al. (3; 50) showed previously that the plasma insulin was higher in male mice compared to female mice in a non-

fasted condition, whereas the difference was not significant in fasted animals. This could explain the lack of any sex difference in our findings until week 10 on HFD. At that time the sex difference in plasma insulin levels was probably pronounced enough to become visible even in fasted animals.

Human studies, however, showed that men had significant higher insulin levels compared to women even in the fasted state (29; 53). The lower insulin levels in women compared to men support our other findings that female mice are less susceptible to obesity (i.e., show lower weight gain and adipose tissue mass) and are more insulin sensitive (i.e., have lower fasting glucose levels and greater glucose tolerance) than male mice when put on HFD.

4.5. Synthesis

Our work shows that when it comes to the mechanisms of HFD-induced obesity and its associated metabolic consequences, sex differences play an important role that should not be underestimated. Female mice differed from male mice already in the first week, and with respect to the first effects of HFD feeding that became obvious, i.e., the body weight gain and adiposity. It is therefore not surprising that sex also influenced the associated metabolic parameters. We also observed sex differences with respect to the effects of the cytokine TNFα on body weight gain and adiposity. The role of TNFα in obesity-related T2D has been thoroughly examined in male animals before, and we extend this work for the first time to females. The observed differences suggest that there is also a sex difference in the inflammatory processes associated with obesity, hence supporting the hypothesis that the mechanisms in the development of obesity and T2D are sex dependent. Although further investigations are necessary to understand the exact mechanisms underlying the effects of sex on the development of diet-induced obesity, our findings indicate that the sex differences should be considered when therapies or preventive measures for are developed which are mostly based on male data.

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